Technical Information

Mueller Hinton Agar

Product Code: DM 1173

Application: Mueller Hinton Agar is used for cultivation of *Neisseria* and for determination of susceptibility of microorganisms to antimicrobial agents.

Composition**

Ingredients	Gms / Litre
Beef, infusion from	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

The Mueller Hinton formulation was originally used as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species [1]. Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing [2]. Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard [3]. Mueller Hinton Agar has been selected by the CLSI for several reasons: as mentioned below.

i. It demonstrates batch-to-batch reproducibility for susceptible testing.
ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors contents.
iii. It supports the growth of most non-fastidious bacterial pathogens and
iv. Much data and experience regarding its performance have been recorded [9] by various works as a scientific proof

For there reasons Kirby-Bauer et al recommended this medium for antibiotic susceptibility tests using a single disc of high concentration [4]. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility [5]. Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae.*

Beef infusion and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazoletrimethoprim (SXT). Calcium and magnesium ion concentrations are adjusted to provide the amounts recommended by CLSI to give the correct MIC values with aminoglycosides and *Pseudomonas aeruginosa* [2].

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values [1, 2, 6]. A standard suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards [7]. The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms [7, 9].

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings [8].

Methodology

Suspend 38 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction
Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH Range
7.2-7.4

Cultural Response/Characteristics
DM 1173: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli *ATCC 25922*	50-100	luxuriant	>=70%
Haemophilus influenzae *ATCC 49247*	50-100	luxuriant(on Mueller Hinton chocolate Agar)	>=70%
Neisseria gonorrhoeae *ATCC 49226*	50-100	luxuriant	>=70%
Pseudomonas aeruginosa *ATCC 27853*	50-100	luxuriant	>=70%
Staphylococcus aureus *ATCC 25923*	50-100	luxuriant	>=70%
Enterococcus faecalis *ATCC 29212*	50-100	luxuriant	>=70%
Streptococcus pneumoniae *ATCC 6305*	50-100	luxuriant(on Mueller Hinton Blood Agar)	>=70%
Escherichia coli *ATCC 35218*	50-100	Luxuriant	>=70%
Staphylococcus aureus *ATCC 43300*	50-100	luxuriant	>=70%

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.
3. NCCLS Approved Standard: ASM-2, 1979, Performance Standards for Antimicrobic disc Susceptibility Tests, 2nd Ed., National Committee for Clin. Lab. Standards.
4. Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.
5. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.

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